Modifying short phenylalanine-phenylalanine peptide sequences to create multifunctional nanomaterials with biomaterial and drug delivery applications

Modifying short FF peptide sequences to create multifunctional nanomaterials with biomaterial and drug delivery applications

Dr Garry Laverty

School of Pharmacy

Biofunctional Nanomaterials Group

Our Research is Funded by

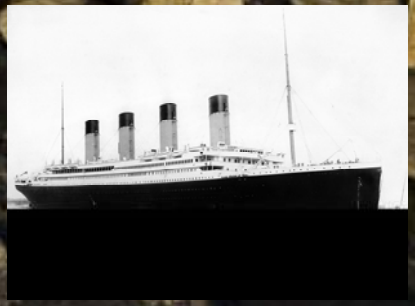
QUEEN'S UNIVERSITY BELFAST

School Of Pharmacy

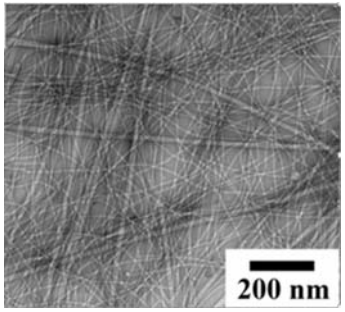
STUDY AT THE SCHOOL

NO. 1 IN THE UK

The School of Pharmacy at Queen's has been ranked as the number 1 school of Pharmacy in the UK

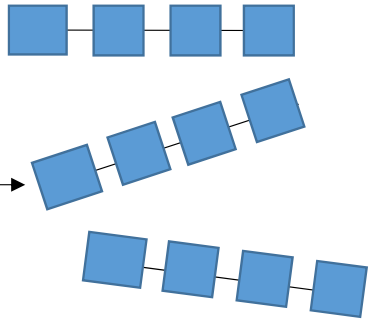


Core Technology



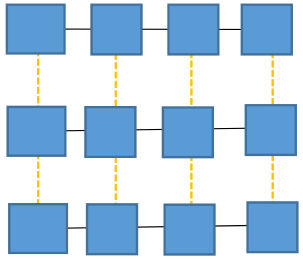
Peptide Hydrogels

Self-assembled Peptides



Stimuli

-pH
-Temperature
-Ionic Strength
-Specific enzymes



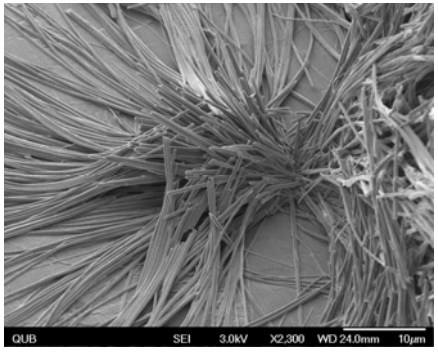
Self-assembly

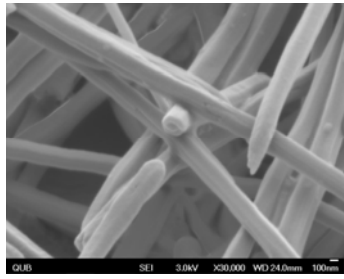
Peptide

Nanotubes

Short peptide sequences

Non assembled





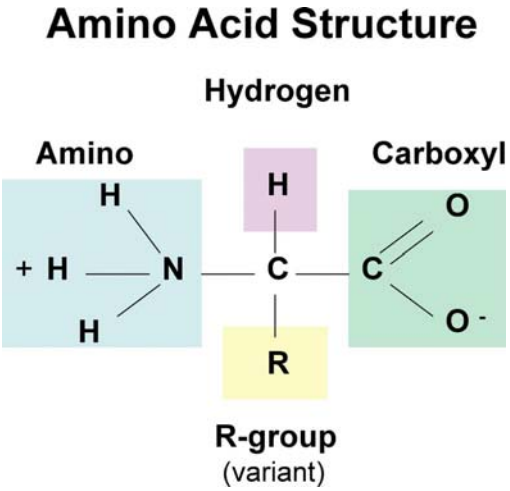
Rational Design of Antimicrobial Peptide Motif vs Self-assembly

Antimicrobial Activity	Propensity to Self-assembled hydrogels
Hydrophobic/Hydrophilic (Charge) ratio (more important with regard to antimicrobial activity than size)	Hydrophobic/Hydrophilic balance
Interactions with microbial extracelluar membranes	Non Covalent intermolecular interactions (e.g. Van der Waal's, π-π stacking)
Interaction with intracellular targets/processes (DNA, RNA, enzymes, protein synthesis). Binds to DNA, lipopolysaccharide to prevent pro-inflammatory response = immunomodulatory	Ability of peptide to form hydrogen bonds with each other and with water

McCloskey A.P., Gilmore, B.F., Laverty, G. (2014) Evolution of Antimicrobial Peptides to Self-Assembled Peptides for Biomaterial Applications. *Pathogens*. *3*(4); 791-821.

Advantages of Ultrashort Peptides

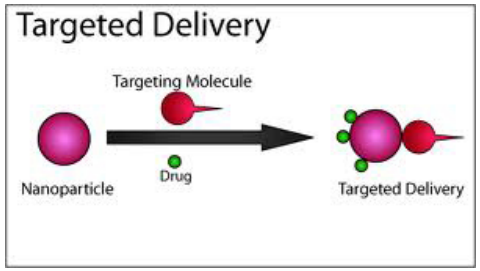
- Successful in producing a series peptide sequences of that self-assemble to form hydrogels or nanotubes in response to physiological stimuli
- Ultrashort peptides (< 7 amino acids) → More cost effective → Upscale by Pharmaceutical Industry → Increased translational potential → Patient benefit
- Numerous advantages over current synthetic materials including:
  - Increased **chemical versatility**
  - Minimal immunogenicity and enhanced biocompatibility
  - Tunable biodegradability
  - Tailored self-assembly/pharmacological properties (e.g. antimicrobial) in response to stimuli



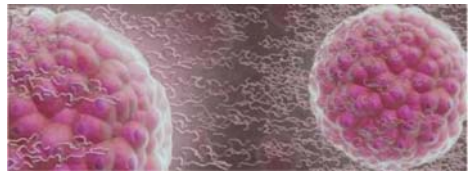
Biofunctional Nanomaterials Utilising the Building Blocks of Life!

Infection and Medical Devices

Wound healing

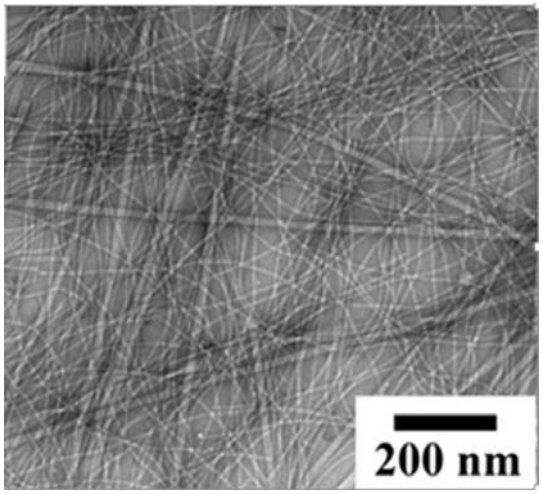


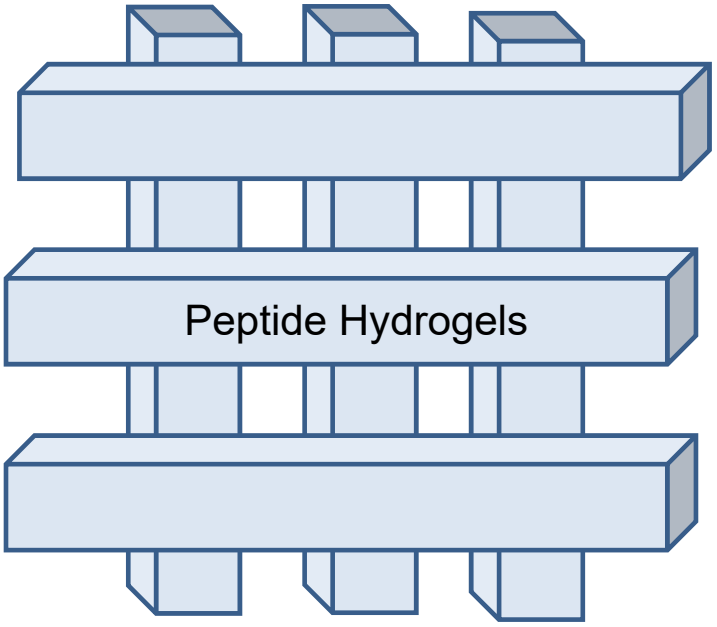
Drug Delivery (Blood brain barrier, cancer, Gram-negative bacteria, HIV, *in situ* forming implants)



Stem Cells/Regenerative medicine

Peptide Hydrogel Nanomaterials

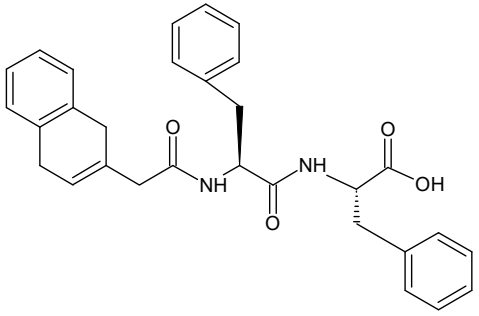




Self-assembled Ultrashort Peptide Gels

- 2013 Research Placement Prof. Bing Xu Lab, School of Chemistry, Brandeis, Waltham, Boston
- Successful in producing a series of ultrashort peptides (< 7 amino acids) that self-assembled at physiological pH
- (X_1-FF-X_2)
- More cost effective
- Hydrophobicity provided by inclusion of a naphthalene (Nap) grouping (at X_1 position) and varying quantity of phenylalanine in primary structure

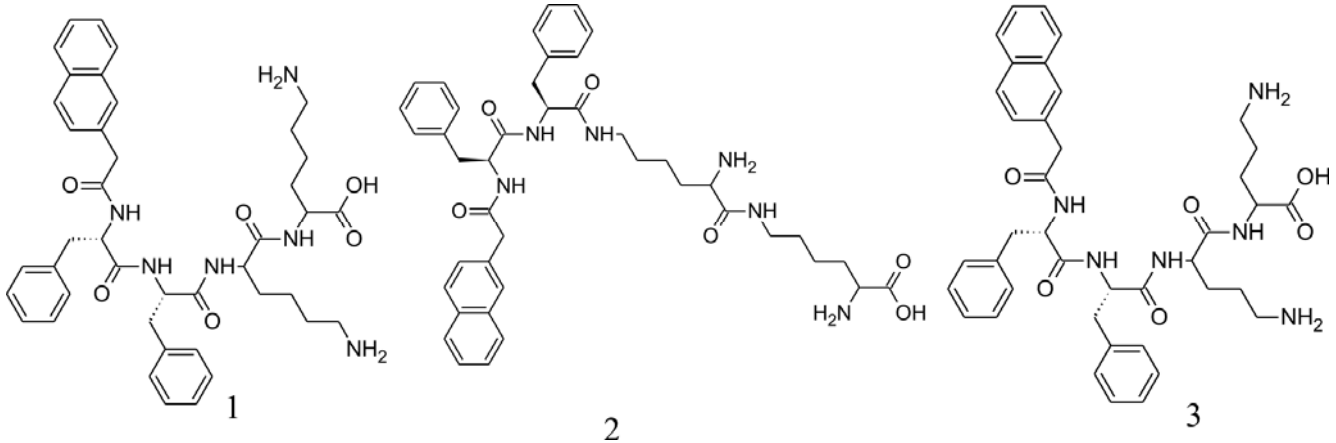
NapFF structure



School Of Pharmacy

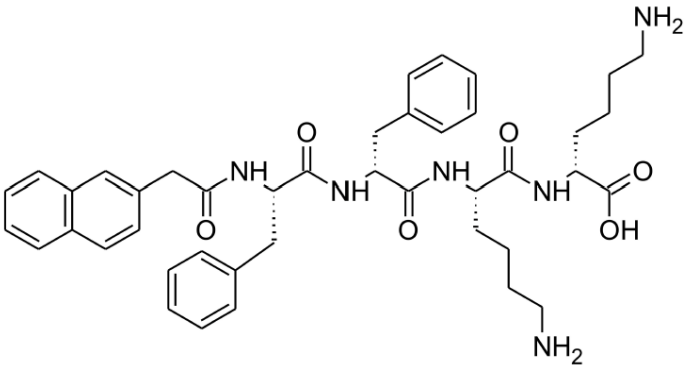
Ultrashort Cationic Variants: Primary Structures

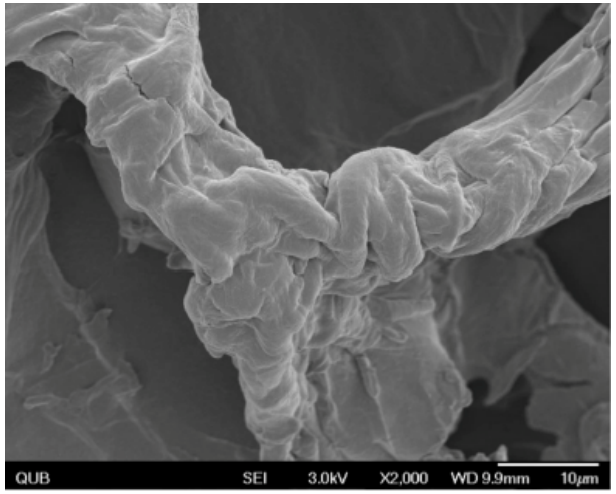
- Charge: Inclusion of cationic amino acids
  - 1) Lysine
  - 2) Ornithine
  - 3) epsilon (ε) Lysine
- Minimum of 2 charged units required for antimicrobial and antibiofilm activity
- Primary amine group provides cationic charge
- Cationic amino acids vary by number of methylene units on R-group

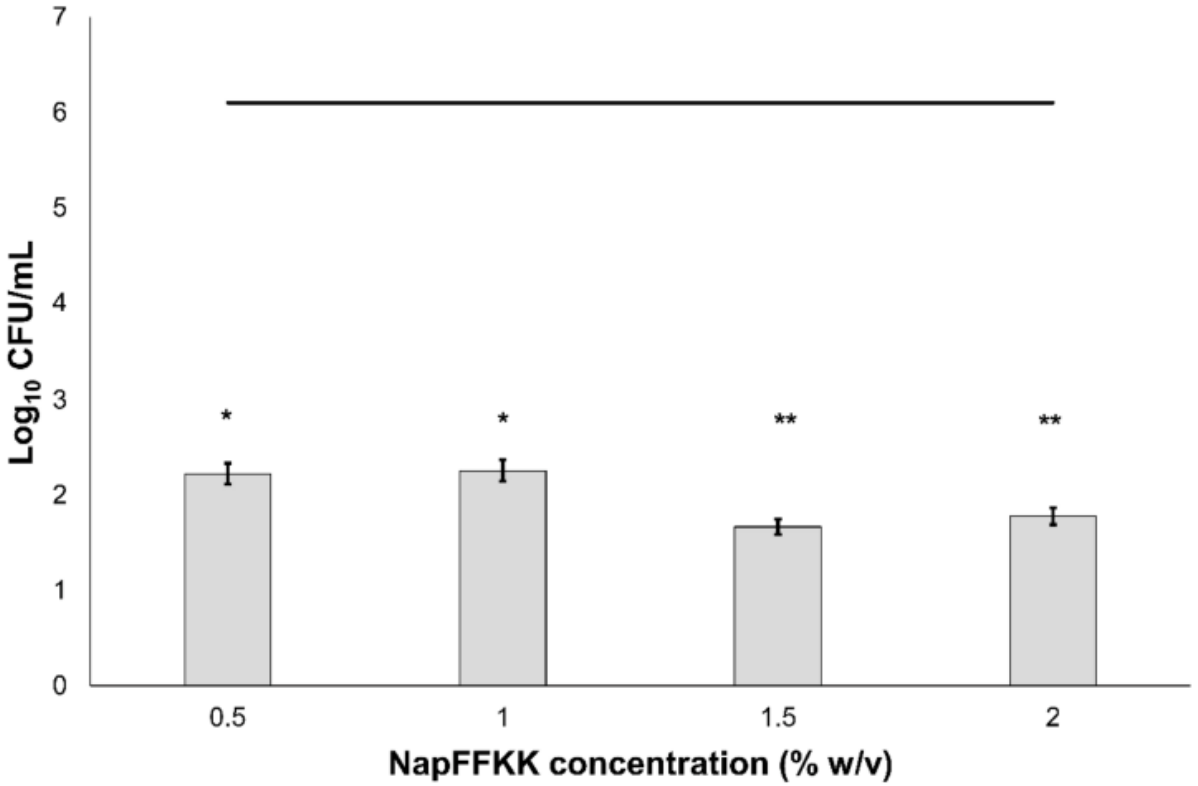


-Laverty, G., McCloskey A.P., Gilmore, B.F., Jones, D.S., Zhou, J., Xu, B (2014). Ultrashort Cationic Naphthalene derived Self-assembled Peptides as Antimicrobial Nanomaterials. *Biomacromolecules*; 15: 3429−3439.

NapFFKK: Fungal infections



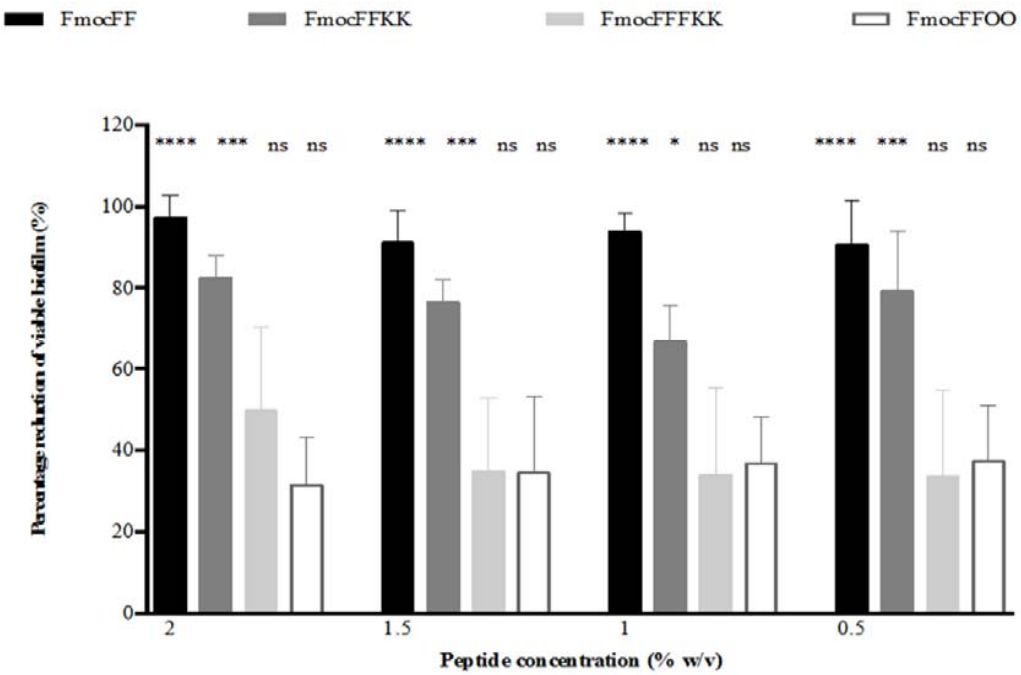




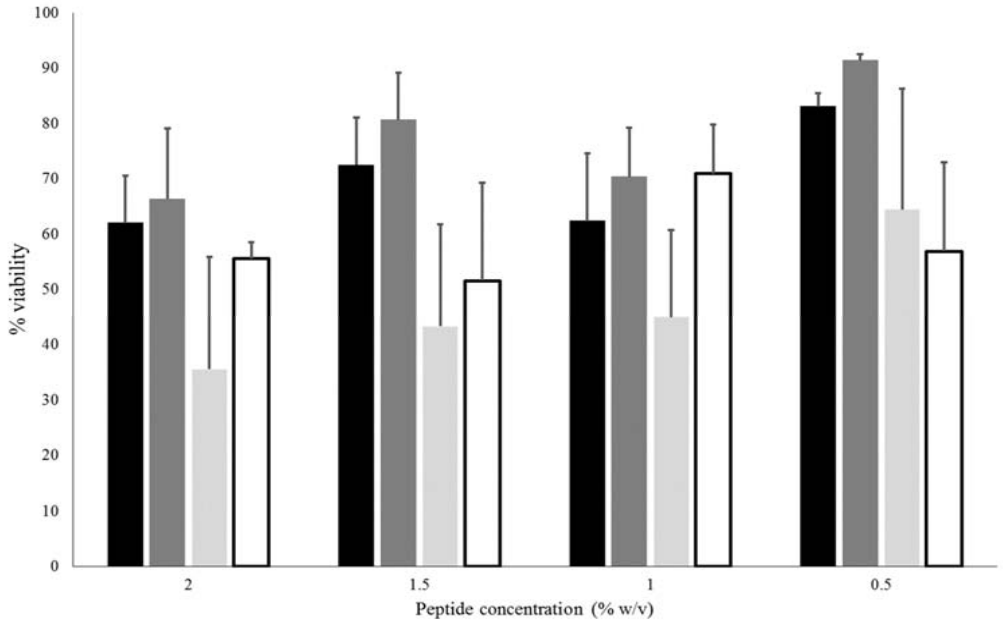
Fungal viability counts (Log10 CFU/mL) of *Aspergillus niger* CABI 017454 after 24 h exposure

- Albadr,A., Gilmore,S.M., Porter,S.L, Thakur,R., Laverty,G.* (2018). Ultrashort self-assembling peptide hydrogel for the treatment of fungal infections. Gels. 4: 48.

Fmoc variants antibiofilm



Percentage reduction in viability of 24 hour *E. coli* (ATCC 11303) biofilm following 24 hour exposure to Fmoc-peptides.

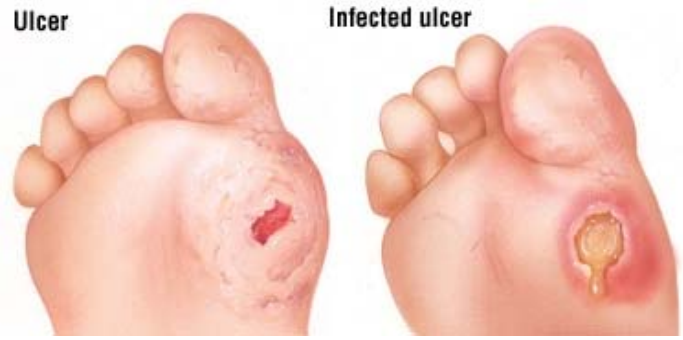


Percentage cell viability of NCTC clone 929 (ATCC CCL 1) cells after 24 hour exposure to varying concentrations of Fmoc-peptides.

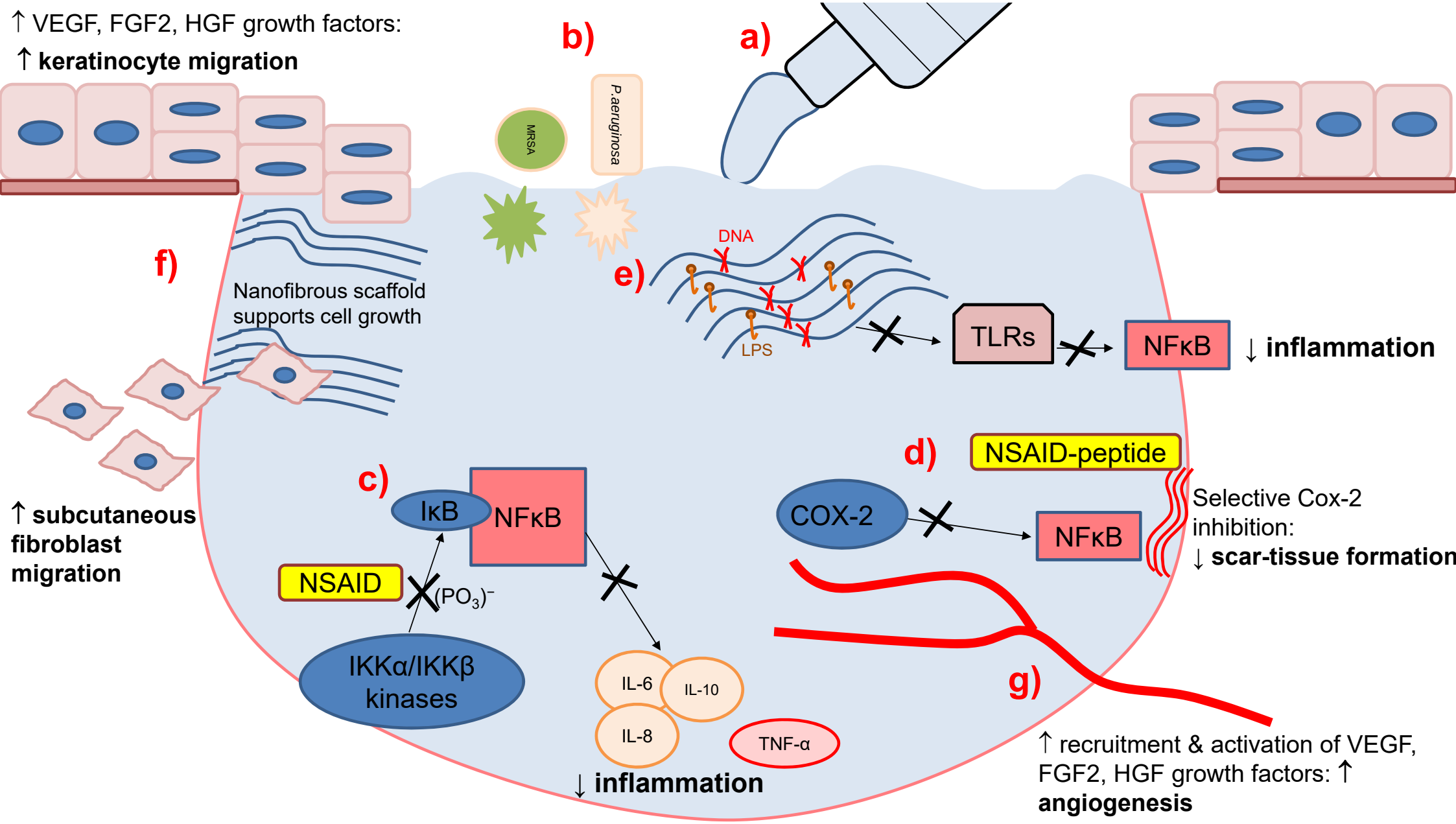
McCloskey,A.P., Draper,E.R., Gilmore, B.F., Laverty,G.* (2017). Ultrashort self-assembling Fmoc-peptide gelators for anti-infective biomaterial applications. Journal of Peptide Science. 23 (2): 131–140.

Multifunctional NSAID-peptide hydrogels for the treatment of chronic wounds

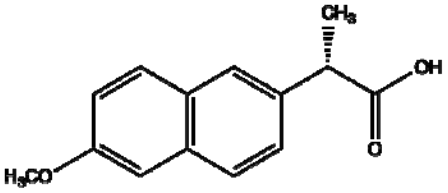
- Chronic wounds: unable to heal fully or respond to treatment within 4 to 12 weeks. E.g. pressure wounds, diabetic ulcers, burn/surgical wounds.
- Latest UK estimates (2005-06), reported an incidence of 575,600 patients annually costing the NHS between £2.3 and 3.1billion, 3% of yearly healthcare expenditure.
- Differ from acute wounds in that they are associated with prolonged inflammation that prevents healing fully: Non steroidal anti-inflammatory drugs (NSAIDs) showing benefit.
- Optimal multifunctional peptide: hydrogelating, biocompatible, antimicrobial, anti-inflammatory, pro-angiogenic



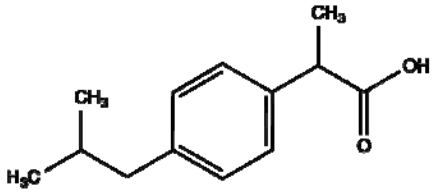
Prevention better than cure!!


↑ VEGF, FGF2, HGF growth factors:
↑ keratinocyte migration
b)
a)
MRSA
P.aeruginosa
f)
Nanofibrous scaffold supports cell growth
e)
DNA
LPS
TLRs
NFκB
↓ inflammation
↑ subcutaneous fibroblast migration
c)
IκB
NFκB
NSAID
(PO3)−
IKKα/IKKβ kinases
IL-6
IL-10
IL-8
TNF-α
↓ inflammation
d)
NSAID-peptide
COX-2
NFκB
Selective Cox-2 inhibition:
↓ scar-tissue formation
g)
↑ recruitment & activation of VEGF, FGF2, HGF growth factors: ↑ angiogenesis

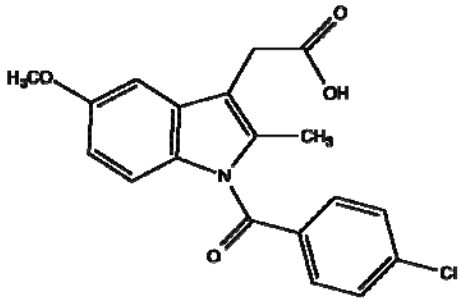
Multifunctional NSAID-peptide hydrogels: Design



Naproxen



Ibuprofen



Indomethacin

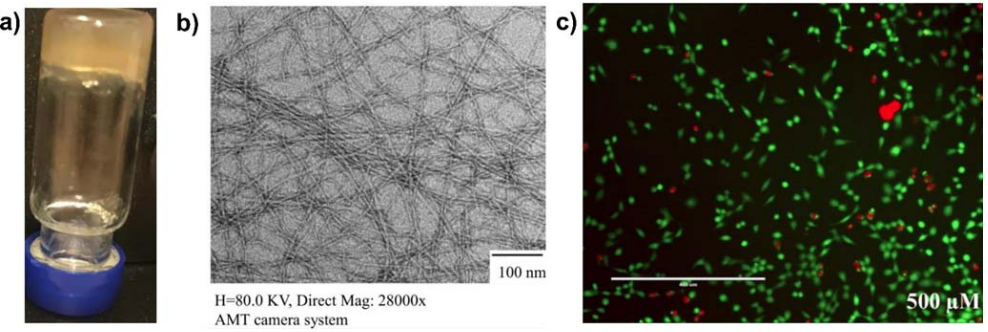
Optimal multifunctional peptide:

- Hydrogelating ✓
- Biocompatible ✓
- Antimicrobial ✓
- Anti-inflammatory/immunomodulatory
  - selective COX-2 inhibition ✓
  - inhibit NFκB
  - inhibit toll-like receptors by binding to biomolecules (e.g. DNA, bacterial LPS)

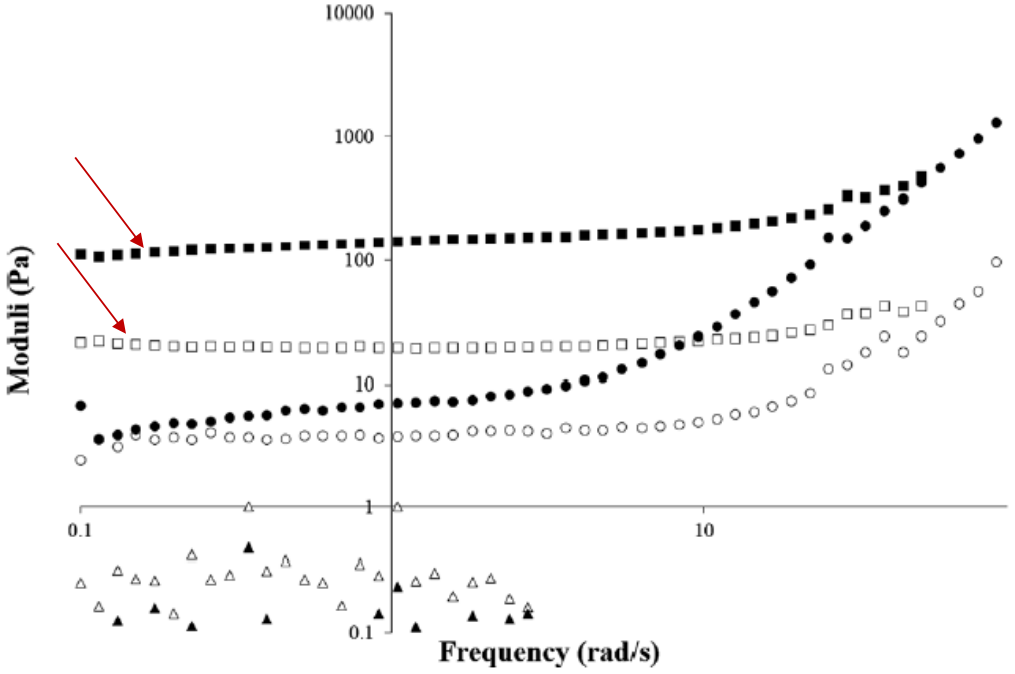
- Pro-angiogenic (heparin mimetic motif)

McCloskey,A.P., Gilmore,S.M., Zhou, J., Draper,E.R., Porter,S., Gilmore, B.F., Xu, B., Laverty,G.* (2016). Self-assembling ultrashort NSAID-Peptide nanosponges: multifunctional antimicrobial and anti-inflammatory materials. RSC Advances. 6: 114738-114749.

Multifunctional NSAID-peptide hydrogels: Hydrogelating, Biocompatible



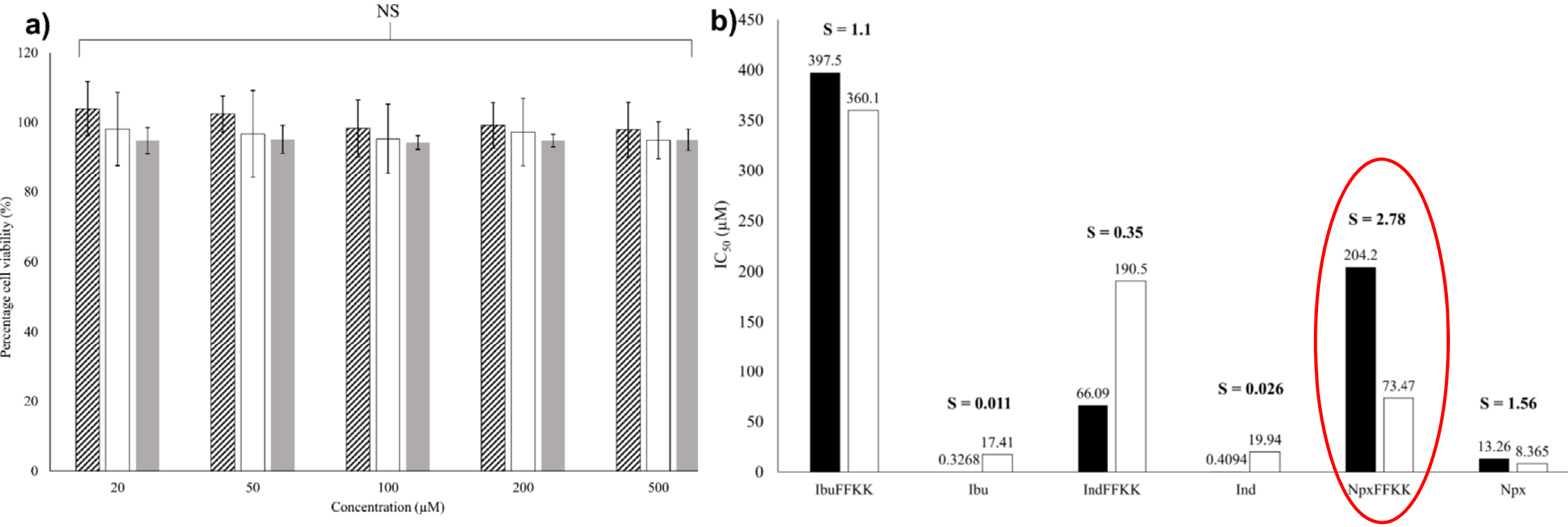
Data relating to L-isomers of 2%w/v peptide. a) Npx-FFKK-OH hydrogel. b) TEM showing Npx-FFKK-OH nanofibres. c) LIVE/DEAD assay, 500µM Npx-FFKK-OH with NCTC929 fibroblasts.



Oscillatory frequency sweep 2% w/v NSAID-peptides. Key: black triangle: G' IbuFFKK, white triangle: G" IbuFFKK black circle: G' IndFFKK, white circle: G" IndFFKK, black square: G' NpxFFKK, white square: G" NpxFFKK.

McCloskey,A.P., Gilmore,S.M., Zhou, J., Draper,E.R., Porter,S., Gilmore, B.F., Xu, B., Laverty,G.* (2016). Self-assembling ultrashort NSAID-Peptide nanosponges: multifunctional antimicrobial and anti-inflammatory materials. RSC Advances. 6: 114738-114749.

Multifunctional NSAID-peptide hydrogels:
Biocompatible & COX-2 selective (anti-inflammatory)

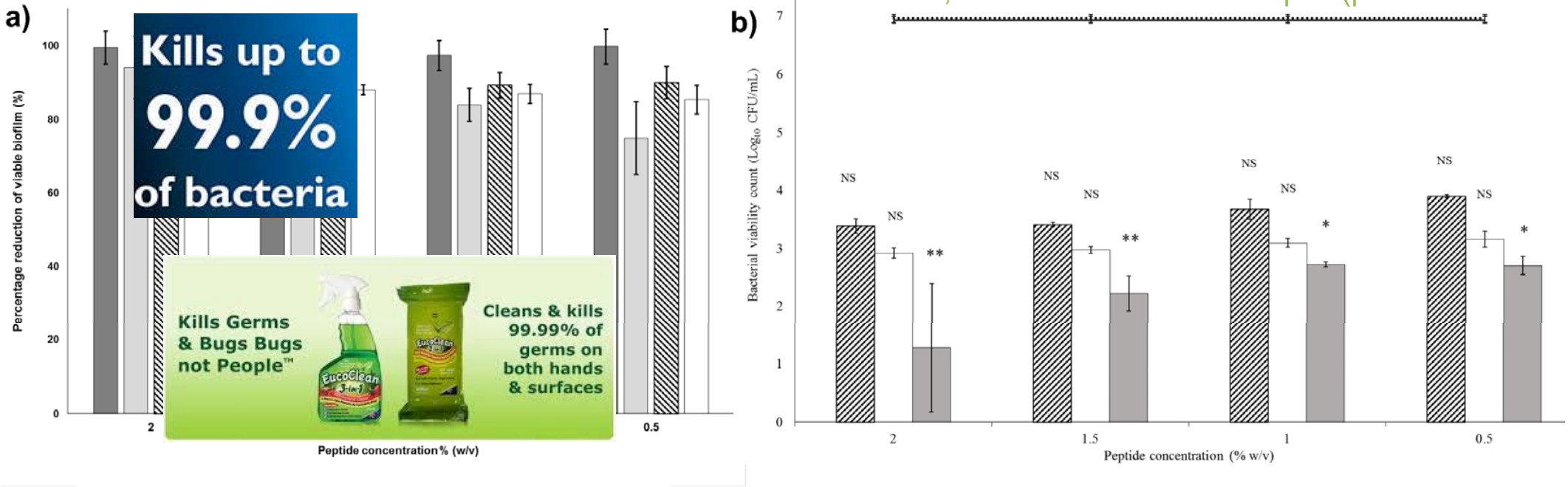


Cell compatibility and COX inhibition of L-isomers of X-FFKK-OH peptides. Ibuprofen (Ibu), indomethacin (Ind) and Npx conjugated at X. **a)** >90% cell viability, NCTC929 fibroblasts, 24 hour exposure (alamar blue assay). Key: striped:IbuFFKK, white:IndFFKK, grey:NpxFFKK, ns:no significant difference compared to negative PBS control. **b)** IC_{50} NSAID-peptide and NSAIDs only, inhibition of COX-1 (black column) and COX-2 (white column). Selectivity (S)=COX-1:COX-2 ratio of IC_{50} values. Addition of FFKK-OH to NSAIDs increases IC_{50} values relative to NSAID only but significant inhibition is maintained within the µM range. NSAID-peptides possess increased COX-2 selectivity compared to NSAID only, which is promising for chronic wound therapy. COX-2 selectivity highest for NpxFFKK-OH (S=2.78) therefore it is the most promising NSAID-peptide for reducing scar tissue formation in chronic wounds.

Multifunctional NSAID-peptide hydrogels: Anti-biofilm/Antimicrobial

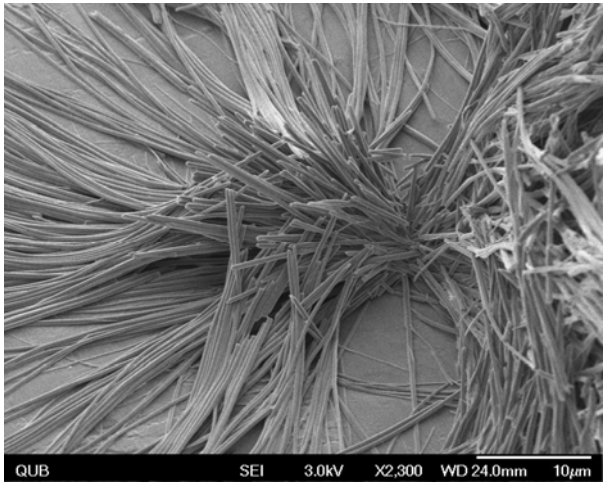
Biofilms

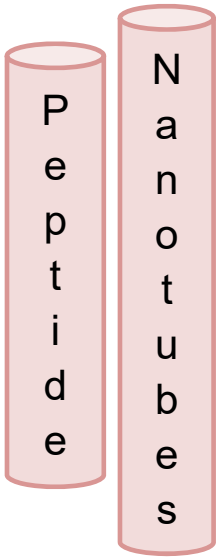
Mixture of biofilms, surface attached and liquid (planktonic bacteria)

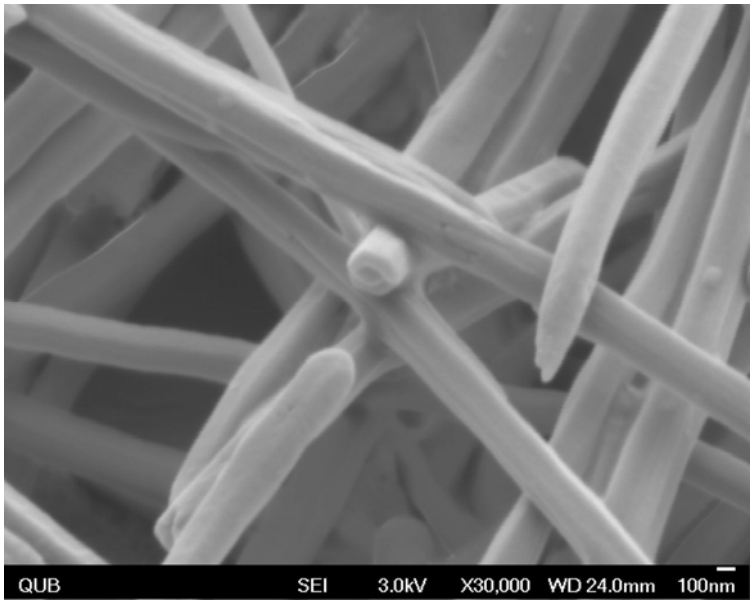


Bactericidal activity of NSAID-FFKK-OH. **a)** NpxFFKK-OH (2-0.5%w/v) shows >90% in 24 hour biofilms of *S.aureus* ATCC25923 (black column), *S.epidermidis* ATCC35984 (grey), *E.coli* ATCC11303 (striped) and *P.aeruginosa* PAO1 (white) after 24 hours (alamar blue assay). **b)** Log_{10} reduction in *S.aureus* viable count after 24 hours, NSAID-peptides (2-0.5%w/v). Key: striped column: IbuFFKK-OH, white: IndFFKK-OH, grey: NpxFFKK-OH, dotted line: PBS control. At least a 3 log_{10}CFU/mL (99.9%) reduction in bacteria, employed as a threshold for efficacy was observed for all NSAID-peptides at concentrations ≥0.5% w/v compared to PBS control. A similar trend was demonstrated for NSAID-peptides against *S.epidermidis*, *E.coli* and *P.aeruginosa*.

Peptide Nanotubes







Tuesday 3rd September ~9.25am: Optimising phenylalanine-phenylalanine peptide nanotubes to demonstrate selective antibiofilm activity

ELSEVIER

Acta Biomaterialia

Volume 77, 1 September 2018, Pages 96-105

Full length article

Self-assembling diphenylalanine peptide nanotubes selectively eradicate bacterial biofilm infection

Simon L. Porter, Sophie M. Coulter, Sreekanth Pentlavalli, Thomas P. Thompson, Garry Laverty

Queen's University Belfast

Thank You!

Department for the Economy

Biofunctional Nanomaterials Group

- Dr Sreekanth Pentlavalli (Wellcome Trust Research Fellow)
- Sophie Gilmore (Dfe funded PhD student): *In situ* implants
- Rawan Huwaitat (PhD student): Selective Gram-negative antimicrobials
- Simon Porter (Dfe funded PhD student) Nanotubes
- Alyaa Albadr (PhD student) Ocular drug delivery/antimicrobial
- Marina Afami (Dfe funded PhD student) Stem cell delivery/dental

http://lavertylab.weebly.com

Our Research is Funded by

society for applied sfam microbiology

EXCELLENCE IN SCIENCE THE ROYAL SOCIETY

BIOCHEMICAL SOCIETY

InterTradeIreland FUSION

wellcome

erasmus

- The Xu Group
  Brandeis University
- The Adams Lab
  University of Glasgow